

IN THE SPECIFICATION:

Please amend page 5, lines 3-5 as follows:

For unregulated expression constitutive promoters are either derived from viral promoters ~~of~~ or
from strong cellular promoters.

Please amend page 5, lines 12-15, to read as follows:

In one aspect the present invention provides a promoter-transactivator system for inducible high-
level mammalian cell gene expression with the option of cell growth control ~~as defined in claim~~
+ comprising

(a) a promoter construct (IRFE promoter) having the general structure:

[MPSV-E]-[IRF-1-binding sites]-[CMV]—DNA, wherein
→ mRNA

MPSV-E means MPSV enhancer repeats of the sequence:

GCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGGAAAAATACATAACTGAGAATAGAGAAG
TTCAGATCAAGGTCAGGAACAGAGAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTA
AGCAGTTCCTGCCCCGCTCAGGGCCAAGAACAGTTGGAACAGGAGAATTGGGCCAAACAGGA
TATCTGTGGTAAGCAGTTCCTGCCCCGCCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGT
CCCGCCCTCAGCAGTTTCTAGA,

or isofunctional variants thereof obtained by substitution, insertion or deletion of one

or more nucleotides,

IRF-1-binding sites means the sequence:

GATCCCTTCTCGGGAAATGGAAACTGAAAATCAGATCCCTTCTCGGGAAATGGAAACTGAAA
ATCAGATC,

or isofunctional variants thereof obtained by substitution, insertion or deletion of one

or more nucleotides, and

CMV means a minimal promoter of the sequence:

TGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAAACCGTCAA
ACCGCGGAAGCT,

or isofunctional variants thereof obtained by substitution, insertion or deletion of one
or more nucleotides,

and

(b) a transactivator construct coding for a fusion protein comprising IRF-1 and the
estrogen receptor.

Please amend page 5, lines 16-22, to read as follows:

In a further aspect the present invention provides an expression vector (s) comprising a promoter
construct and/or a transactivator construct as defined ~~in claim 1~~ above (i.e. said promoter
construct and said transactivator construct may be incorporated in the same expression vector
thus containing said a promoter-transactivator system or may be incorporated in separate
expression vectors).

Please amend page 5, lines 25-27, to read as follows:

In yet another aspect the present invention provides a process for inducible high-level
mammalian gene expression with the option of cell growth control ~~as defined in claim 4~~
comprising the steps of:

(a) transfecting or transforming mammalian cells with the expression vector of claim

2;

(b) culturing said mammalian cells in a suitable medium; and,

BB Vance

Please amend page 6, lines 2-4 as follows:

2.

Please amend page 6, lines 23-26 as follows:

27

Please amend page 6, lines 31-35 as follows:



Please add the following on page 9, line 13:

B9

The invention will now be described in further detail with respect to the accompanying

in which:

B9
care

Figure 1 shows a regulated promoter that shows a higher maximal secreted pharmaceutical protein productivity than a contemporary highly efficient viral promotor (MPSV, myeloproliferative sarcoma virus). The artificial promoter consists of viral enhancer repeats (MSPV), of IRF-1-binding sites (ISRE-luc, Interferon Stimulated Response Element-firefly luciferase: Kichhoff and Hauser, 1999) and of a viral minimal promoter element (CMV, cytomegalo virus).

Please delete page 14, lines 5 -page 15 line 8 as follows:

~~Examples for specific applications in industrial relevant systems.~~

~~Figures 10 and 11 relate to two examples of a relevant pharmaceutical protein-expressing BHK (baby hamster kidney) cell clones with IRF-hER enhanced productivity (pg/cell/day, E2) versus control (pg/cell/day, ctrl) and reduced proliferation (living cells x 10e5/ml, E2) versus control (living cells, ctrl).~~

~~The following is one example for increased IgG antibody productivity in a long term experiment in IRF-hER proliferation controlled BHK 21 cells in a perfused fermenter with microcarriers showing also high viability. For additional characterization of lesser importance glucose consumption and lactate production was determined.~~

~~Name: Fermenter 3~~

Conditions: ~~IgG 8 cells, Cytodex 3 microcarrier, 6g/l, medium for all fermenters: DMEM: F12 with 5% serum and 500 ug/ml G418 and 3 ug/ml Puro (with added dextran and Pluronic)~~

Fermenter: ~~New Brunswick, stirred tank, 5 l tank capacity, 3.5 l working volume, settling tube as separator (a glass tube in which the microcarriers settle down by gravitational action) while the medium is removed. Works effective up to a perfusion rate of 1 volume/day (also 3.5 l/day). For higher values gravitational action is not sufficient to compensate sucking action.~~

At the end of the induction stirring was stopped for 20 min. After the microcarriers have settled down the medium was removed by suction except for 0.5 l, added up to 2 l (=wash), again removed by suction except for 0.5 l and added up to 3.5 l. Approximately 7% of the initial content of E2 remains (since, however, during induction perfusion was activated, the actual value for E2 was below 7%). The washing procedure took about 1-1.5 h.

In all fermenters the perfusion rate was in the range from 0 to 0.9, wherein most of the time the rate was in the range 0.6 to 0.9.

Please add the following on page 15, line 12:

DETAILED DESCRIPTION OF THE INVENTION

Examples for specific applications in industrial relevant systems.

Figures 10 and 11 show the results of two examples of a relevant pharmaceutical protein expressing BHK (baby hamster kidney) cell clones with IRF-hER enhanced productivity (pg/cell/day, E2) versus control (pg/cell/day, ctrl) and reduced proliferation (living cells x 10e5/ml, E2) versus control (living cells, ctrl).

The following is one example for increased IgG antibody productivity in a long-term experiment in IRF-hER proliferation controlled BHK-21 cells in a perfused fermenter with microcarriers showing also high viability. For additional characterization of lesser importance glucose consumption and lactate production was determined (Figure 12).

Name: Fermenter 3

Conditions: IgG 8 cells, Cytodex 3 microcarrier, 6g/l, medium for all fermenters: DMEM: F12 with 5% serum and 500 ug/ml G418 and 3 µg/ml Puro (with added dextran and Pluronic).

Fermenter: New Brunswick, stirred tank, 5 l tank capacity, 3.5 l working volume, settling tube as separator (a glass tube in which the microcarriers settle down by gravitational action) while the medium is removed. Works effective up to a perfusionrate of 1 volume/day (also 3.5 l/day). For higher values gravitational action is not sufficient to compensate sucking action.

At the end of the induction stirring was stopped for 20 min. After the microcarriers have settled down the medium was removed by suction except for 0.5 l, added up to 2 l (=wash), again removed by suction except for 0.5 l and added up to 3.5 l. Approximately 7% of the initial

content of E2 remains (since, however, during induction perfusion was activated, the actual value for E2 was below 7%). The washing procedure took about 1-1.5 h.

B10
correct
In all fermenters the perfusion rate was in the range from 0 to 0.9, wherein most of the time the rate was in the range 0.6 to 0.9.

Please amend page 15, line 9 as follows:

B10
Figure 12 shows the results, of an example for increased IgG antibody productivity in a long-term experiment in IRF-hER proliferation controlled BHK-21 cells in a perfused fermenter with microcarriers showing also high viability. For additional characterization of lesser importance glucose consumption and lactate production was determined.

Please amend page 22, line 1 as follows:

Claims

We claim: